NEW BACILLUS THURINGIENSIS STRAINS AND THEIR INSECTICIDAL PROTEINS

This invention relates to four novel strains of *Bacillus thuringiensis* (the "BTS02617A strain", the "BTS02618A strain", the "BTS02654B strain" and the "BTS02652E strain"), each of which produces crystallized proteins (the "BTS02617A crystal proteins", the "BTS02618A crystal proteins", the "BTS02654B crystal proteins" and the "BTS02652E crystal proteins", respectively) which are packaged in crystals (the "BTS02617A crystals", the "BTS02618A crystals", the "BTS02654B crystals" and the "BTS02652E crystals", respectively) during sporulation. The BTS02617A, BTS02618A, BTS02654B and BTS02652E strains were deposited under the provisions of the Budapest Treaty at the Belgian Coordinated Collections of Microorganisms - Collection Laboratorium voor Microbiologie Belgium ("BCCM-LMG"), R.U.G., K. Ledeganckstraat 35, B-9000 Gent.

This invention also relates to an insecticide composition that is active against Lepidoptera and that comprises the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, as such, or preferably the BTS02617A, BTS02618A, BTS02654B or BTS02652E crystals, crystal proteins or the active component(s) thereof as an active ingredient.

This invention further relates to a gene (the "*bTS02618A* gene"), which is present in the genome of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains and which encodes an insecticidal protein (the "BTS02618A protoxin") that is found in the BTS02617A, BTS02618A, BTS02654B and BTS02652E crystals. The BTS02618A protoxin is the protein that is produced by the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains before being packaged into their respective BTS02617A, BTS02618A, BTS02654B and BTS02652E crystals.

This invention still further relates to a toxin (the "BTS02618A toxin") which can be obtained (e.g., by trypsin digestion) from the BTS02618A protoxin. The BTS02618A toxin is an insecticidally active protein which can be liberated from

the BTS02617A crystals, the BTS02618A crystals, the BTS02654B crystals, and the BTS02652E crystals, which are produced by the BTS02617A strain, the BTS02618A strain, the BTS02654B strain and the BTS02652E strain, respectively. This toxin and its protoxin have a high activity against a wide range of lepidopteran insects, particularly against Noctuidae, especially against _Spodoptera_ and _Agrotis_ spp., but also against other important lepidopteran insects such as Pyralidae, particularly the European corn borer, _Ostrinia nubilalis_, and Yponomeutidae such as _Plutella xylostella_. This new characteristic of the BTS02618A protoxin and toxin ("(pro)toxin"), i.e., the combination of activity against different economically important Lepidopteran insect families such as Noctuidae, Yponomeutidae and Pyralidae, makes this (pro)toxin an ideally suited compound for combatting a wide range of insect pests by contacting these insects with the (pro)toxin, e.g., by spraying or by expressing the bTS02618A gene in plant-associated bacteria or in plants. The BTS02618A toxin is believed to represent the smallest portion of the BTS02618A protoxin which is insecticidally effective against Lepidoptera.

This invention yet further relates to a chimeric gene that can be used to transform a plant cell and that contains the following operably linked DNA fragments:

1) a part of the _bTS02618A_ gene (the "insecticidally effective _bTS02618A_ gene part") encoding an insecticidally effective portion of the BTS02618A protoxin, preferably a truncated part of the _bTS02618A_ gene (the "truncated _bTS02618A_ gene") encoding just the BTS02618A toxin;
2) a promoter suitable for transcription of the insecticidally effective _bTS02618A_ gene part in a plant cell; and
3) suitable 3' end transcript formation and polyadenylation signals for expressing the insecticidally effective _bTS02618A_ gene part in a plant cell.

This chimeric gene is hereinafter generally r ferred to as the "_bTS02618A_ chimeric gene".

This invention also relates to:

1) a cell (the "transformed plant cell") of a plant, such as corn or cotton, the genome of which is transformed with the insecticidally effective bTS02618A gene part, preferably the bTS02618A chimeric gene; and

2) a plant (the "transformed plant") which is regenerated from the transformed plant cell or is produced from the so-regenerated plant and their seeds, the genome of which contains the insecticidally effective bTS02618A gene part, preferably the bTS02618A chimeric gene, and which is resistant to Lepidoptera.

This invention still further relates to :

1) a microbial organism, such as *B. thuringiensis* or *Pseudomonas* spp., the genome of which is transformed with all or part of the bTS02618A gene; and

2) a microbial spore, containing a genome which is transformed with all or parts of the bTS02618A gene.

Background of the Invention

B. thuringiensis ("Bt") is a Gram-positive bacterium which produces endogenous crystals upon sporulation. The crystals are composed of proteins which are specifically toxic against insect larvae. These crystal proteins and corresponding genes have been classified based on their structure and insecticidal spectrum (Höfte and Whiteley, 1989). The four major classes are Lepidoptera-specific (cryI), Lepidoptera- and Diptera-specific (cryII), Coleoptera-specific (cryIII), and Diptera-specific (cryIV) genes.

The fact that conventional submerged fermentation techniques can be used to produce Bt spores on a large scale makes Bt bacteria commercially attractive as a source of insecticidal compositions.

Gene fragments from some Bt strains, encoding insecticidal proteins, have heretofore been identified and integrated into plant genomes in order to render the plants insect-resistant. However, obtaining expression of such Bt gene fragments in

plants is not a straightforward process. In order to achieve optimal expression of an insecticidal protein in plant cells, it has been found necessary to engineer each Bt gene fragment in a specific way so that it encodes a part of a Bt protoxin that retains substantial toxicity against its target insects (European patent application ("EPA") 86/300,291.1 and 88/402,115.5; U.S. patent application 821,582, filed January 22, 1986).

Summary of the Invention

In accordance with this invention, four novel Bt strains, i.e., the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains, are provided. The BTS02617A, BTS02618A, BTS02654B and BTS02652E crystals and crystal proteins, the BTS02618A protoxin and toxin produced by the strains during sporulation, and insecticidally effective portions of the BTS02618A protoxin, as well as equivalents of these crystals, crystal proteins, protoxin, toxin and insecticidally effective protoxin portions, each possess insecticidal activity and can therefore be formulated into insecticidal compositions against Lepidoptera in general, and particularly against Noctuidae, such as Agrotis spp. (cutworms such as Agrotis ipsilon), Mamestra spp. (e.g., the cabbage moth, Mamestra brassica) and Spodoptera spp. (armyworms, such as Spodoptera exigua, Spodoptera frugiperda, Spodoptera littoralis and Spodoptera litura), against Pyralidae (e.g., the European corn borer, Ostrinia nubilalis) and Yponomeutidae (such as Plutella xylostella) which are major pests of various economically important crops, such as corn, cotton and many vegetables such as Brassicas.

Also in accordance with this invention, a plant cell genome is transformed with the insecticidally effective bTS02618A gene part, preferably the truncated bTS02618A gene, or an equivalent thereof such as a modified, synthetic bTS02618A gene. It is preferr d that this transformation be carried out with the bTS02618A chimeric gene. The resulting transformed plant cell can be used to produce transformed

plants, seeds of transformed plants and plant cell cultures consisting essentially of the transformed cells. The transformed cells in some or all of the tissues of the transformed plants: 1) contain the insecticidally effective bTS02618A gene part as a stable insert in their genome, and 2) express the insecticidally effective bTS02618A gene part by producing an insecticidally effective portion of its BTS02618A protoxin, preferably its BTS02618A toxin, thereby rendering the plant resistant to Lepidoptera. The transformed plant cells of this invention can also be used to produce, for recovery, such insecticidal Bt proteins.

Further in accordance with this invention, a process is provided for rendering a plant resistant to Lepidoptera by transforming the plant cell genome with the insecticidally effective bTS02618A gene part, preferably the truncated bTS02618A gene, or an equivalent thereof. In this regard, it is preferred that the plant cell be transformed with the bTS02618A chimeric gene.

Yet further in accordance with this invention, there are provided the BTS02618A protoxin, the insecticidally effective portions of such protoxin and the BTS02618A toxin, as well as functional parts of the BTS02618A toxin, as well as the bTS02618A gene, the insecticidally effective bTS02618A gene part, the truncated bTS02618A gene and the chimeric bTS02618A gene, as well as their equivalents.

Also in accordance with this invention, a DNA sequence, either natural or artificial, encoding the BTS02618A protoxin or insecticidally effective portions thereof, such as the toxin, is provided.

Also in accordance with this invention are provided an insecticidal composition against Lepidoptera, particularly Noctuidae, Pyralidae and Yponomeutidae, and a method for controlling Lepidoptera, particularly Noctuidae, Pyralidae and Yponomeutida , with the insecticidal composition, wherein the insecticidal composition comprises the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, crystals and/or crystal proteins or the BTS02618A protoxin, toxin and/or insecticidally

effective protoxin portions or their equivalents.

Detailed Description of the Invention

The BTS02618A protoxin of this invention can be isolated in a conventional manner from the BTS02617A strain, deposited on July, 2 at the BCCM-LMG under accession number **LMG P-12592**, the BTS02618A strain, deposited on July 2, 1992 at the BCCM-LMG under accession number **LMG P-12593**, the BTS02654B strain, deposited on July 2, 1992 at the BCCM-LMG under accession number **LMG P-12594**, or the BTS02652E strain deposited on March 1, 1993 at the BCCM-LMG under accession number **LMG P-13493**. For example, the BTS02617A, BTS02618A, BTS02654B or BTS02652E crystals can be isolated from sporulated cultures of their respective strain (Mahillon and Delcour, 1984), and then, the BTS02618A protoxin can be isolated from the crystals according to the method of Höfte et al. (1986). The protoxins can be used to prepare monoclonal or polyclonal antibodies specific for the protoxin in a conventional manner (Höfte et al., 1988). The BTS02618A toxin can be obtained by protease (e.g., trypsin) digestion of the BTS02618A protoxin.

The bTS02618A gene can be isolated in a conventional manner. The bTS02618A gene can be identified in the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, using the procedure described in U.S. Patent Application 821,582, filed January 22, 1986, and in EPA 86/300,291.1 and 88/402,115.5 (which are incorporated herein by reference). The bTS02618A gene was identified by: digesting total DNA from one of the above strains with restriction enzymes; size fractionating the DNA fragments, so produced, into DNA fractions of 5 to 10 Kb; ligating these fractions to cloning vectors; screening the *E. coli*, transformed with the cloning vectors, with a DNA probe that was constructed from a region of the cryIG gene (Smulevitch et al., 1991; Gleave et al., 1992).

The term "bTS02618A gene" as used herein includes a DNA sequence encoding the BTS02618A protoxin or toxin or functionally equivalent variants thereof. Indeed, because of

th degeneracy of th genetic code, som amino acid codons can be r placed with others without changing the amino acid sequence of the protein. Furthermore, some amino acids can be substituted by other equivalent amino acids without significantly changing the insecticidal activity of the protein. Also, changes in amino acid composition in regions of the molecule, different from those responsible for binding and toxicity are less likely to cause a difference in insecticidal activity of the protein. Such equivalents of the gene include DNA sequences hybridizing to the DNA sequence of the BTS02618A toxin or protoxin of SEQ ID. No. 4 and encoding a protein with the same insecticidal characteristics as the BTS02618A (pro)toxin, of this invention. In this context, the term "hybridization" refers to conventional hybridization conditions, most preferably stringent hybridization conditions.

The term "functional parts of the BTS02618A toxin" as used herein means any part(s) or domain(s) of the toxin with a specific structure that can be transferred to another (Bt) protein for providing a new hybrid protein with at least one functional characteristic (e.g., the binding and/or toxicity characteristics) of the BTS02618A toxin (Ge et al., 1991). Such parts can form an essential feature of the hybrid Bt protein with the binding and/or toxicity characteristics of the BTS02618A protein. Such a hybrid protein can have an enlarged host range, an improved toxicity and/or can be used in a strategy to prevent insect resistance development (European Patent Publication ("EP") 408 403; Visser et al., 1993).

Alternatively, the 5 to 10 Kb fragments, prepared from total DNA of the BTS02617A or BTS02618A or BTS02654B or BTS02652E strain, can be ligated in suitable expression vectors and transformed in _E. coli_, and the clones can then be screened by conventional colony immunoprobing methods (French et al., 1986) for expression of the toxin with monoclonal or polyclonal antibodies raised against the BTS02618A toxin.

Also, the 5 to 10 Kb fragments, prepared from total DNA of the BTS02617A or BTS02618A or BTS02654B or BTS02652E strain, can be ligated in suitable Bt shuttle vectors (Lereclus et al.,

1992) and transformed in a crystal minus Bt-mutant. The clones are then screened for production of crystals (detected by microscopy) or crystal proteins (detected by SDS-PAGE).

The so-identified bTS02618A gene was sequenced in a conventional manner (Maxam and Gilbert, 1980) to obtain the DNA sequence. Hybridization in Southern blots and sequence comparison indicated that this gene is different from previously described genes encoding protoxins and toxins with activity against Lepidoptera (Höfte and Whiteley, 1989).

An insecticidally effective part of the bTS02618A gene, encoding an insecticidally effective portion of its protoxin, and a truncated part of the gene, encoding just its toxin, can be made in a conventional manner after sequence analysis of the gene. The amino acid sequence of the BTS02618A protoxin and toxin was determined from the DNA sequence of the bTS02618A gene and the truncated bTS02618A gene. By "an insecticidally effective part" or "a part" of the bTS02618A gene is meant a DNA sequence encoding a polypeptide which has fewer amino acids than the BTS02618A protoxin but which is still toxic to Lepidoptera.

In order to express all or an insecticidally effective part of the bTS02618A gene or an equivalent gene in *E. coli*, in other Bt strains and in plants, suitable restriction sites can be introduced, flanking each gene or gene part. This can be done by site-directed mutagenesis, using well-known procedures (Stanssens et al., 1989; White et al., 1989). In order to obtain improved expression in plants, it may be preferred to modify the codon usage of the bTS02618A gene or insecticidally effective bTS02618A gene part to form an equivalent, modified or artificial gene or gene part in accordance with PCT publications WO 91/16432 and WO 93/09218; EP 0,358,962 and EP 0,359,472. For obtaining enhanced expression in monocot plants such as corn, a monocot intron also can be added to the bTS02618A chimeric gene, and the DNA sequence of the bTS02618A g ne part can be further changed in a translationally neutral manner, to modify possibly inhibiting DNA sequences present in the g ne part by means of site-

direct d intron insertion and/or by introducing changes to the codon usage, e.g., adapting the codon usage to that most preferred by the specific plant (Murray et al., 1989) with ut changing significantly the encoded amino acid sequence.

The insecticidally effective bTS02618A gene part or its equivalent, preferably the bTS02618A chimeric gene, encoding an insecticidally effective portion of the BTS02618A protoxin, can be stably inserted in a conventional manner into the nuclear genome of a single plant cell, and the so-transformed plant cell can be used in a conventional manner to produce a transformed plant that is insect-resistant. In this regard, a disarmed Ti-plasmid, containing the insecticidally effective bTS02618A gene part, in Agrobacterium tumefaciens can be used to transform the plant cell, and thereafter, a transformed plant can be regenerated from the transformed plant cell using the procedures described, for example, in EP 0,116,718, EP 0,270,822, PCT publication WO 84/02,913 and European Patent Application ("EPA") 87/400,544.0 (which are also incorporated herein by reference), and in Gould et al. (1991). Preferred Ti-plasmid vectors each contain the insecticidally effective bTS02618A gene part between the border sequences, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as direct gene transfer (as described, for example in EP 0,233,247), pollen mediated transformation (as described, for example in EP 0,270,356, PCT publication WO 85/01856, and US Patent 4,684,611), plant RNA virus-mediated transformation (as described, for example in EP 0,067,553 and US Patent 4,407,956), liposome-mediated transformation (as described, for example in US Patent 4,536,475), and other methods such as the recently described methods for transforming certain lines of corn (Fromm et al., 1990 ; Gordon-Kamm et al., 1990) and ric (Shimamoto et al., 1989; Datta et al., 1990) and the recently described method for transforming monocots generally (PCT publication WO 92/09696).

The resulting transformed plant can be used in a

conventional plant breeding scheme to produce more transformed plants with the same characteristics or to introduce the insecticidally effective bTS02618A gene part in other varieties of the same or related plant species. Seeds, which are obtained from the transformed plants, contain the insecticidally effective bTS02618A gene part as a stable genomic insert. Cells of the transformed plant can be cultured in a conventional manner to produce the insecticidally effective portion of the BTS02618A protoxin, preferably the BTS02618A toxin, which can be recovered for use in conventional insecticide compositions against Lepidoptera (U.S. Patent Application 821,582; EPA 86/300291.1.).

The insecticidally effective bTS02618A gene part, preferably the truncated bTS02618A gene, is inserted in a plant cell genome so that the inserted gene is downstream (i.e., 3') of, and under the control of, a promoter which can direct the expression of the gene part in the plant cell. This is preferably accomplished by inserting the bTS02618A chimeric gene in the plant cell genome. Preferred promoters include: the strong constitutive 35S promoters (the "35S promoters") of the cauliflower mosaic virus of isolates CM 1841 (Gardner et al., 1981), CabbB-S (Franck et al., 1980) and CabbB-JI (Hull and Howell, 1987); and the TR1' promoter and the TR2' promoter (the "TR1' promoter" and "TR2' promoter", respectively) which drive the expression of the 1' and 2' genes, respectively, of the T-DNA (Velten et al., 1984). Alternatively, a promoter can be utilized which is not constitutive but rather is specific for one or more tissues or organs of the plant (e.g., leaves and/or roots) whereby the inserted bTS02618A gene part is expressed only in cells of the specific tissue(s) or organ(s). For example, the insecticidally effective bTS02618A gene part could be selectively expressed in the leaves of a plant (e.g., corn, cotton) by placing the insecticidally effective gene part under the control of a light-inducible promoter such as the promoter of the ribulose-1,5-bisphosphate carboxylase small subunit gen of the plant itself or of another plant such as pea as disclosed in U.S. Patent Application 821,582 and EPA

86/300,291.1. Anoth r alternativ is to use a promoter whose expression is inducible (e.g., by temperature or chemical factors).

The insecticidally effective bTS02618A gene part is inserted in the plant genome so that the inserted gene part is upstream (i.e., 5') of suitable 3' end transcription regulation signals (i.e., transcript formation and polyadenylation signals). This is preferably accomplished by inserting the bTS02618A chimeric gene in the plant cell genome. Preferred polyadenylation and transcript formation signals include those of the octopine synthase gene (Gielen et al., 1984) and the T-DNA gene 7 (Velten and Schell, 1985), which act as 3'-untranslated DNA sequences in transformed plant cells.

The insecticidally effective bTS02618A gene part can optionally be inserted in the plant genome as a hybrid gene (EPA 86/300,291.1; Vaeck et al., 1987) under the control of the same promoter as a selectable marker gene, such as the neo gene (EP 0,242,236) encoding kanamycin resistance, so that the plant expresses a fusion protein.

All or part of the bTS02618A gene, encoding an anti-lepidopteran protein, can also be used to transform other bacteria, such as a B. thuringiensis which has insecticidal activity against Lepidoptera or Coleoptera. Thereby, a transformed Bt strain can be produced which is useful for combatting a wide spectrum of lepidopteran and coleopteran insect pests or for combatting additional lepidopteran insect pests. Transformation of bacteria with all or part of the bTS02618A gene, incorporated in a suitable cloning vehicle, can be carried out in a conventional manner, preferably using conventional electroporation techniques as described in Mahillon et al. (1989) and in PCT Patent publication WO 90/06999.

The BTS02617A, BTS02618A, BTS02654B or BTS02652E strain also can be transformed with all or an insecticidally effective part of one or more foreign Bt genes such as: the bt18 gene (EP 0,358,557) or another Bt gene coding for an anti-Lepidoptera protein; and the bt109P g ne (PCT publication WO 91/16433),

coding for an anti-Coleoptera protein. Thereby, a transformed Bt strain can be produced which is useful for combatting an even greater variety of insect pests (e.g., Coleoptera and/or additional Lepidoptera).

Transformation of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain with all or part of a foreign Bt gene, incorporated in a conventional cloning vector, can be carried out in a well known manner, preferably using conventional electroporation techniques (Chassy et al., 1988) or other methods, e.g., as described by Lereclus et al. (1992).

Each of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strains can be fermented by conventional methods (Dulmage, 1981; Bernhard and Utz, 1993) to provide high yields of cells. Under appropriate conditions which are well understood (Dulmage, 1981), the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains each sporulate to produce crystal proteins containing the BTS02168A protoxin in high yields.

An insecticidal, particularly anti-lepidopteran, composition of this invention can be formulated in a conventional manner using the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain or preferably their respective crystals, crystal proteins or the BTS02168A protoxin, toxin or insecticidally effective protoxin portion as an active ingredient, together with suitable carriers, diluents, emulsifiers and/or dispersants (e.g., as described by Bernhard and Utz, 1993). This insecticide composition can be formulated as a wettable powder, pellets, granules or dust or as a liquid formulation with aqueous or non-aqueous solvents as a foam, gel, suspension, concentrate, etc. The concentration of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, crystals, crystal proteins, or the BTS02618A protoxin, toxin or insecticidally effective protoxin portions in such a composition will depend upon the nature of the formulation and its intended mode of use. G nerally, an insecticide composition of this invention can be used to protect a field for 2 to 4 weeks against Lepidoptera with each application of the composition. For more extended protection (e.g., for a whole

growing season), additional amounts of the c mposition should be applied periodically.

A method for controlling insects, particularly Lepidoptera, in accordance with this invention preferably comprises applying (e.g., spraying), to a locus (area) to be protected, an insecticidal amount of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, spores, crystals, crystal proteins or the BTS02168A protoxin, toxin or insecticidally effective protoxin portions, preferably the BTS2168A toxin. The locus to be protected can include, for example, the habitat of the insect pests or growing vegetation or an area where vegetation is to be grown.

To obtain the BTS02618A protoxin or toxin, cells of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain can be grown in a conventional manner on a suitable culture medium and then lysed using conventional means such as enzymatic degradation or detergents or the like. The protoxin can then be separated and purified by standard techniques such as chromatography, extraction, electrophoresis, or the like. The toxin can then be obtained by trypsin digestion of the protoxin.

The BTS02617A, BTS02618A, BTS02654B or BTS02652E cells can also be harvested and then applied intact, either alive or dead, preferably dried, to the locus to be protected. In this regard, it is preferred that a purified BTS02617A, BTS02618A, BTS02654B or BTS02652E strain (either alive or dead) be used, particularly a cell mass that is 90.0 to 99.9 % of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain.

The BTS02617A, BTS02618A, BTS02654B, or BTS02652E cells, crystals or crystal proteins or the BTS02618 protoxin, toxin, or insecticidally effective protoxin portion can be formulated in an insecticidal composition in a variety of ways, using any number of conventional additives, wet or dry, depending upon the particular use. Additives can include wetting agents, detergents, stabilizers, adhering agents, spreading agents and extenders. Examples of such a composition include pastes, dusting powders, wettable powders, granules, baits and aerosol

sprays. Other Bt cells, crystals, crystal proteins, protoxins, toxins, and insecticidally effective protoxin porti ns and other insecticides, as well as fungicides, biocides, herbicides and fertilizers, can be employed along with the BTS02617A, BTS02618A, BTS02654B or BTS02652E cells, crystals or crystal proteins or the BTS02618 protoxin, toxin or insecticidally effective protoxin portions to provide additional advantages or benefits. Such an insecticidal composition can be prepared in a conventional manner, and the amount of the BTS02617A, BTS02618A, BTS02654B or BTS02652E cells, crystals or crystal proteins or the BTS02618A protoxin, toxin or insecticidally effective protoxin portion employed depends upon a variety of factors, such as the insect pest targeted, the composition used, the type of area to which the composition is to be applied, and the prevailing weather conditions. Generally, the concentration of the BTS02618A protoxin, insecticidally effective protoxin portions or toxin will be at least about 0.1% by weight of the formulation to about 100% by weight of the formulation, more often from about 0.15% to about 0.8% by weight of the formulation.

In practice, some insects can be fed the BTS02618A protoxin, toxin, insecticidally effective protoxin portion or mixtures thereof in the protected area, that is in the area where such protoxin, toxin and/or insecticidally effective protoxin portion has been applied. Alternatively, some insects can be fed intact and alive cells of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain or transformants thereof, so that the insects ingest some of the strain's protoxin and suffer death or damage.

The following Examples illustrate the invention. The figure and the sequence listing referred to in the Examples are as follows:

Figure 1

Southern blot analysis of AluI-digested total DNA of Bt strain HD127 (lane 1), the BTS02618A strain (lane 2), Bt strain

BTS02459 (containing cryIA(c), 81k, cryIC en cryIE, lan 3), and Bt strain BTS02480E (containing the same genes as HD-127, lane 4), using a mixture of DNA-probes for cryI crystal protein genes, including the cryIG probe (SEQ ID no. 1). Each band corresponds to a particular crystal protein gene. With these probes, the BTS02618A strain is found to contain the cryIA(b) gene and a novel gene, which is the bTS02618A gene, identified by an AluI fragment of approximately 530 bp, hybridizing to the cryIG probe of SEQ ID no. 1. The names of the recognized cryI genes are indicated, as well as the size of some fragments. The bTS02618A gene is indicated with three asterisks; "?" indicates an unknown gene fragment.

Sequence Listing

SEQ ID No. 1 - Nucleotide sequence of the DNA probe used to isolate the bTS02618A gene. This probe is derived from part of the cryIG DNA sequence and is complementary to nucleotides 2732-2750 of the DNA sequence described by Smulevitch et al. (1991).

SEQ ID No. 2 - The 5' partial nucleotide sequence of the bTS02618A gene, comprising the presumptive translation initiation codon at nucleotide position 195-197.

SEQ ID No. 3 - The 3' partial nucleotide sequence of the bTS02618A gene (N: unknown nucleotide), comprising the presumptive translational stop codon at nucleotide position 1146-1148.

SEQ ID No. 4 - The nucleotide sequence of the bTS02618A gene and the translated amino acid sequence of the BTS02618A protoxin. The open reading frame of the protoxin reaches from nucleotide 668 to nucleotide 4141. The translation initiation codon is at nucleotide position 668-670, the translation stop codon is at nucleotide position 4139-4141.

Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA are carried out by the standardized procedures described in Sambrook et al.,

Molecular Cloning - A Laboratory Manual, Second Ed., Cold Spring Harbor Laboratory Press, NY (1989).

Example 1: Characterization of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains.

The BTS02617A, the BTS02618A and the BTS02654B strain were isolated from grain dust sampled in Cadlan, province of Bicol, The Philippines and were deposited at the BCCM-LMG on July 2, 1992 under accession Nos. LMG P-12592, LMG P-12593 and LMG P-12594, respectively. Strain BTS02652E was also isolated from Philippine grain dust, and was deposited at the BCCM-LMG on March, 1, 1993 under accession No. LMG P-13493.

Each strain can be cultivated on conventional standard media, preferably T_3 medium (tryptone 3 g/l, tryptose 2 g/l, yeast extract 1.5 g/l, 5 mg $MnCl_2$, 0.05 M Na_2PO_4, pH 6.8 and 1.5% agar), preferably at 28°C. For long term storage, it is preferred to mix an equal volume of a spore-crystal suspension with an equal volume of 50% glycerol and store this at -70°C or lyophilize a spore-crystal suspension. For sporulation, growth on T_3 medium is preferred for 48 hours at 28°C, followed by storage at 4°C. During its vegetative phase, each of the strains can also grow under facultative anaerobic conditions, but sporulation only occurs under aerobic conditions.

Sterilization of each strain occurs by autoclave treatment at 120°C (1 bar pressure) for 20 minutes. Such treatment totally inactivates the spores and the BTS02617A,BTS02618A, BTS02654B, and BTS02652E protoxins. UV radiation (254 nm) also inactivates the spores.

After cultivating on Nutrient Agar ("NA", Difco Laboratories, Detroit, MI, USA) for one day, colonies of each of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains form opaque white colonies with irregular edges. Cells of each strain (Gram positive rods of 1.7-2.4 x 5.6-7.7 μm) sporulate after 48 hrs cultivation at 28°C on T_3 agar. The crystal proteins produced during sporulation are packaged in crystals of the BTS02617A, BTS02618A, BTS02654B, and BTS02652E strains.

Quite remarkably, the crystal remains attached to the spore after sporulation.

The Bt serotype of the BTS02617A, BTS02618A, BTS02645B and BTS02652E strains was determined to be serotype tolworthi H9 of all these strains which was determined by conventional serotyping methods as conducted by the WHO Collaborating Center for Entomopathogenic Bacillus.

Example 2 : Insecticidal activity of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains and the BTS02618A protoxin against Noctuidae spp., Yponomeutidae spp. and Pyralidae spp.

Toxicity assays were performed on neonate larvae (for *Plutella xylostella*, third instar larvae were used) fed on an artificial diet layered with spore-crystal mixtures from one of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains or the BTS02618A protoxin or toxin. The artificial diet was dispensed in wells of Costar 24-well plates. Formaldehyde was omitted from the diet. 50 μl of a sample dilution was applied on the surface of the diet and dried in a laminar air flow. For LC_{50} assays, the dilutions were made in a PBS-BSA buffer, and five dilutions were applied. Two larvae were placed in each well and 24 larvae were used per sample dilution. Dead and living *M. brassica*, *S. frugiperda*, *H. virescens*, *O. nubilalis*, *Plutella xylostella* and *S. exigua* larvae were counted on the fifth day, and dead and living *A. ipsilon* and *S. littoralis* larvae were counted on the sixth day. The LC_{50} and LC_{95} values (the concentrations required to kill respectively 50% or 95% of the insects tested, expressed in number of spore-crystals/cm^2 or ng (pro)toxin/cm^2) were calculated using Probit-analysis (Finney, 1971), and the results are set forth below.

<u>Spodoptera litt ralis</u>

Experiment/Strain	LC_{50} [a]	LC_{95} [a]	$FL_{min-max}$ [b]	Slope
Experiment 1				
BTS02618A	2.4	7.7	1.5-3.4	3.2
HD127[c]	2.5	168	1.2-7.4	1.0
Experiment 2				
BTS02618A	1.1	4	0.8-1.6	3.0
HD127	21.2	133.7	14.4-31.9	2.0

[a] 10^5 spore-crystals per cm^2

[b] 95 % fiducial limits of LC_{50} values

[c] from the Howard Dulmage collection, housed at the Northern Region Research Center, 1815 North University, Peoria, Ill, USA. The curator is Dr. L.Nakamura.

Experiments with purified BTS02618A protoxin also show a significant toxicity of this protoxin against <u>S. littoralis</u> larvae.

Spodoptera exigua

1. Crystal/spore mixtures

Experiment/Strain	LC_{50} [a]	LC_{95} [a]	$FL_{min-max}$ [b]	Slope
Experiment 1				
BTS02618A	1.4	7.9	0.48-3.9	2.2
HD127	8.2	163.5	5.1-15.7	1.3
Experiment 2				
BTS02618A	1.2	3.56	0.91-1.57	3.5
BTS02617A	0.79	2.12	0.61-1.03	3.81
HD127	3.5	44.2	1.36-11.5*	1.5
Florbac	4.1	53.9	1.5-17.0*	1.47
BTS00170U[c]	5.1	46.5	1.83-24.4*	1.71
Experiment 3				
Javelin[d]	23.12	195.7	14.6-56.7	1.77
Experiment 4				
BTS02618A	1.07	2.91	0.83-1.39	3.8
BTS02617A	0.87	4.7	0.59-1.21	2.22
HD127	4.7	56.9	1.85-18.7*	1.52
Florbac[e]	2.53	48.1	0.79-6.71*	1.29
BTS00170U	1.94	56.3	0.55-5.4*	1.12

[a] 10^5 spore-crystals per cm^2

[b] 95 % fiducial limits of LC_{50} values, values marked with * are 90 % fiducial limits of LC_{50} values

[c] PCT patent publication WO 90/06999

[d] strain isolated from Javelin (Sandoz, Lichtstrasse, Basel, Switzerland)

[e] strain from Florbac (Novo Nordisk, Novo Allè, Bagsværd, Denmark)

2. Toxin/protoxin assays.

ICP		LC_{50}[a]	LC_{95}[a]	$FL_{min-max}$[b]	Slope
BTS02618A	Protoxin	26.6	100.6	20.9-33.9	2.8
CryIC	Toxin	68.9	313.2	50.5-94.1	2.5
CryID	Toxin	118.6	870.6	82.7-170.0	1.9

[a] ng/cm^2
[b] 95 % fiducial limits of LC_{50} values

<u>**Mamestra brassica**</u>

1. Crystal/spore mixtures.

Experiment/Strain	LC_{50}[a]	LC_{95}[a]	$FL_{min-max}$[b]	Slope
HD127	37.8	297.6	17.8-91.1	1.8
BTS02618A	8.6	59.6	6.0-12.2	1.9
BTS02617A	5.2	25.8	3.7-7.1	2.4
BTS02652E	12.9	44.2	9.7-17.2	3.0
BTS02654B	14.2	60.5	10.8-19.9	2.6

[a] 10^5 spore-crystals per cm^2
[b] 95 % fiducial limits of LC_{50} values

2. Protoxin assays.

ICP		LC_{50}[a]	LC_{95}[a]	$FL_{min-max}$[b]	Slop
BTS02618A	Protoxin	25.3	125.1	19.3-33.2	2.4
CryIC	Protoxin	22.0	62.9	16.3-29.6	3.6
CryIA(b)	Protoxin	162.4	7169	93.2-283.1	1.0

[a] ng/cm^2
[b] 95 % fiducial limits of LC_{50} values

Agr tis ipsil n

1.Crystal/spore mixtures.

Strain	mortality[a]	genes[b]
Btgall.[c]	1/20	cryIF, cryIG, cryII, 81k
HD127[d]	2/20	cryIAa, cryIAb, cryIC, cryID, cryII, 81k
BTS02618A	16/20[e]	cryIAb, cryII, bTS02618A
Buffer	1/20	none

[a] number of 1st instar larvae killed after 6 days (10^7 spore-crystals per cm^2)
[b] genes known to be present in these strains
[c] Btgall. as described by Smulevitch et al (1991)
[d] HD127 is available at the Howard Dulmage Collection (NRRC, see above)
[e] surviving larvae show severe growth-inhibition

STRAIN	LC_{50}[a]	LC_{95}[a]	$FL_{min-max}$[b]	Slope
BTS02618A	84.4	207.9	65.9-109.6	4.2
HD127	>250			
BTS02617A	53.4	261.0	27.7-112.3	2.4

[a] 10^6 spores/cm^2
[b] 95 % fiducial limits of LC_{50} values

2. Toxin/protoxin assay.

ICP		LC_{50}[a]	LC_{95}[a]	$FL_{min-max}$[b]	Slope
CryIAc	Toxin	>1350			
BTS02618A	Protoxin	212.2	1973	168.1-267.9	1.7

[a] ng/cm^2
[b] 95 % fiducial limits of LC_{50} values

Since MacIntosh t al. (1990) described som activity of the CryIAc toxin towards A. ipsilon, purified CryIAc toxin was tested on this insect for comparison but did not cause any significant mortality of A. ipsilon.

Heliothis virescens

1.Crystal/spore mixture.

Experiment/Strain	LC_{50}[a]	LC_{95}[a]	$FL_{min-max}$[b]	Slope
BTS02617A	1.69	14.99	0.67-2.89	1.73
BTS02618A	2.71	25.4	0.88-6.99	1.69
BTS00170U[c]	15.1	398.7	8.3-41.2	1.15
Dipel[d]	2.99	14.11	1.25-7.76	2.45

[a] 10^3 spore-crystals per cm^2
[b] 95% fiducial limits of LC_{50} values
[c] PCT patent publication WO 90/06999
[d] strain isolated from Dipel™ (Abbott Laboratories, North Chicago, Ill., USA)

2.Toxin/protoxin assay.

ICP		LC_{50}[a]	$FL_{min-max}$[b]	LC_{95}[a]	Slope
BTS02618A	Protoxin	31.6	20-50	182.7	2.1
CryIAb	Toxin	7.2	4.9-10.5	169.1	1.2

[a] ng/cm2
[b] 95 % fiducial limits of LC_{50} values

<u>Ostrinia nubilalis</u>

1. Crystal/spore mixtures.

Experiment/Strain	LC_{50}[a]	LC_{95}[a]	$FL_{min-max}$[b]	Slope
BTS02617A	4.92	12.49	2.45-6.81	4.0
BTS02618A	6.17	39.7	2.93-9.74	2.0
Dipel[c]	>30			

[a] 10^5 spore-crystals per cm^2
[b] 95% fiducial limits of LC_{50} values
[c] strain isolated from Dipel™ (Abbott Laboratories)

2. Purified protoxin assay

ICP		100 % Mortality[a]
CryIAb	Toxin	1350
CryIB	Toxin	1350
BTS02618A	Protoxin	100

[a] concentration at which 100 % mortality was observed (in ng/cm^2)

The purified BTS02618A protoxin also showed a significant toxicity to <u>Ostrinia nubilalis</u> larvae, as compared with the CryI toxins that are most active against <u>Ostrinia</u>.

<u>Plutella xylostella</u>

<u>Plutella xylostella</u> larvae also showed significant mortality after application of purified BTS02618A toxin to their artificial diet in several experiments.

Sp doptera frugiperda

Crystal/spore mixtures of a bTS02618A gene-transformed crystal-minus Bt strain (Mahillon et al., 1989) were also found to significantly inhibit larval growth of S. frugiperda larvae in insect feeding trials.

In conclusion, the strains of this invention and the BTS02618A protein of this invention have a strong insecticidal activity against a broad range of insects that are not susceptible to any single currently available Bt protein and have an activity against at least three Spodoptera spp. and against other Noctuidae, such as A. ipsilon, M. brassica and H. virescens, as well as against Pyralidae, such as O. nubilalis and Yponomeutidae such as Plutella xylostella. These results are summarized and compared with results for other CryI genes (Van Frankenhuyzen, 1993) in Table 1 which shows the unique range of insects susceptible to the BTS02618A protein.

Example 3 : Identification of the bTS02618A gene

The bTS02618A gene was identified in the BTS02618A strain by Southern blot analysis (Fig. 1) of AluI digested total DNA of the strain using, as a DNA probe, the DNA sequence of the cryIG gene (Gleave et al., 1992) of SEQ ID No. 1 and using standard hybridization conditions. Partial DNA sequences of the bTS02618A gene, showing its 5' and 3' end portions, are shown in SEQ ID Nos. 2 and 3, respectively, and the full DNA sequence of the bTS02618A gene and the full amino acid sequence of the BTS02618A protein are shown in SEQ ID No. 4.

The partial sequences of SEQ ID Nos. 2 and 3 allow the bTS02618A gene to be recognized in the BTS02617A, BTS02654B and BTS02652E strains and allow the construction of probes to identify and isolate the full gene sequence in these and other Bt strains. The translation initiation codon of the bTS02618A gene is identified at nucleotide position 195-197 in SEQ ID No. 2, corresponding to nucleotide position 668-670 in SEQ ID No.4. The translation stop codon is identified at nucleotide position 1146-1148 in SEQ ID No. 3, corresponding to nucleotide position

4139-4141 in SEQ ID No. 4.

Th bTS02618A gene was also identified in the BTS02617A, BTS02654B and BTS02652E strains by using the DNA sequence of SEQ ID No. 1 as a probe, as well as other DNA probes of conserved DNA fragments in cryI genes.

The full length bTS02618A gene was found to encode a 129.9 kD protoxin. A comparison of the amino acid sequence with other known CryI proteins showed that the C-terminal part (C-terminal of conserved sequence block 5) was homologous with CryIG (88%). The best homology for the N-terminal part (the toxin) was found with the CryIB toxin, but this was found to be less than 50% (homology is expressed as the number of perfect matches divided by the number of amino acids of the longest fragment).

The smallest insecticidal protein is believed to be a 69 kD (615 amino acids) protein stretching from amino acid number 44 to amino acid number 658 in SEQ ID No. 4. A smaller tryptic fragment of 55 kD (494 amino acids), stretching from amino acid number 165 to amino acid number 658 in SEQ ID No. 4, still has insecticidal activity towards *S. exigua*, but this activity is significantly reduced. Thus, a truncated bTS02618A gene or an equivalent truncated gene preferably encodes the 69 kD protein of the BTS02618A protoxin of SEQ ID No.4 as described above.

Example 4 : Cloning and expression of the bTS02618A gene

In order to isolate the bTS02618A gene, total DNA from the BTS02618A strain was prepared and partially digested with *Sau*3A. The digested DNA was size fractionated on a sucrose gradient and fragments ranging from 7 Kb to 10 Kb were ligated to the *Bam*H1-digested and BAP-treated cloning vector pUC19 (Yannisch-Perron et al., 1985). Recombinant *E.coli* clones containing the vector were then screened with the cryIG DNA probe of SEQ ID No. 1 which is described in Example 3, to identify clones containing the bTS02618A gene.

The so-identified DNA fragments were then sequenced according to Maxam and Gilbert (1980). Partial sequences of the bTS02618A gene are shown in SEQ ID Nos. 2 and 3, and a full

sequence of the <u>bTS02618A</u> gene and the BTS02618A protein is shown in SEQ ID No. 4. Based on the DNA sequence analysis, the gene is cut with appropriate restriction enzymes to give the truncated <u>bTS02618A</u> gene encoding the BTS02618A toxin. Expression of the gene in <u>E.coli</u> was induced using standard procedures (Sambrook et al., 1989, <u>supra</u>).

The <u>bTS02618A</u> gene is also introduced by routine procedures into a crystal-minus Bt strain, using Bt plasmids PGI2 or PGI3 (Mahillon and Seurinck 1988; Mahillon et al., 1988).

<u>Example 5: Insertion of the bTS02618A gene and the truncated bTS02618A gene in E. coli and insertion of the truncated bTS02618A gene in plants.</u>

In order to express the <u>bTS02618A</u> gene and the truncated <u>bTS02618A</u> gene of Example 4 in <u>E. coli</u> and in plants, different gene cassettes are made in <u>E. coli</u> according to the procedure described in EPA 86/300291.1 and EPA 88/402115.5.

To allow significant expression in plants, cassettes containing a) the truncated gene or b) a hybrid gene that is a fusion of i) the truncated gene and ii) the <u>neo</u> gene are each: inserted between the T-DNA border sequences of intermediate plant expression vectors as described in EPA 86/300291.1; fused to transcript formation and polyadenylation signals in the plant expression vectors; placed under the control of the constitutive promoter from cauliflower mosaic virus driving the 35S3 transcript (Hull and Howell, 1987) or the 2' promoter from the TR-DNA of the octopine Ti-plasmid (Velten et al., 1984); and fused to 3' end transcript formation and polyadenylation signals of the octopine synthase gene (Gielen et al., 1984).

Using standard procedures (Deblaere et al., 1985), th intermediate plant expression vectors, containing the truncated <u>bTS02618A</u> gene, are transferred into the <u>Agrobacterium</u> strain C58C1Rif^R (US Patent Application 821,582; EPA 86/300,291.1) carrying th disarmed Ti-plasmid pGV2260 (Vaeck et al., 1987). Selection for spectinomycin resistance yields cointegrated

plasmids, consisting of pGV2260 and the respective intermediate plant expression vectors. Each of these recombinant Agrobacterium strains is then used to transform different cotton plants so that the truncated bTS02618A gene is contained in, and expressed by, different plant cells.

Example 6: Expression of the truncated bTS02618A gene in plants.

The insecticidal activity against Lepidoptera of the expression products of the truncated bTS02618A gene in leaves of transformed plants, generated from the transformed plant cells of Example 5, is evaluated by recording the growth rate and mortality of Agrotis and Spodoptera spp. larvae fed on these leaves. These results are compared with the growth rate of larvae fed leaves from untransformed plants. Toxicity assays against Agrotis and Spodoptera spp. are performed as described in EP 0,358,557, U.S. Patent Application 821,582 and EPA 86/300,291.1. A significantly higher mortality rate is obtained among larvae fed on leaves of transformed plants containing the truncated bTS02618A gene and the truncated bTS02618A-neo hybrid gene than among larvae fed the leaves of untransformed plants. The transformed plants are also found to resist Ostrinia nubilalis Mamestra brassica, Heliothis virescens and Plutella xylostella attack by their expression of the BTS02618A protein.

Needless to say, this invention is not limited to the BTS02617A strain (BCCM-LMG P-12592), the BTS02618A strain (BCCM-LMG P-12593), the BTS02654B strain (BCCM-LMG P-12594) and the BTS02652E (BCCM-LMG P-13493) strain. Rather, the invention also includes any mutant or variant of the BTS02617A, BTS02618A, BTS02654B, and BTS02652E strain which produces crystals, crystal proteins, protoxin or toxin having substantially the same properties, particularly anti-Lepidoptera properties, quite particularly anti-Noctuidae, anti-Yponomeutidae and anti-Pyralidae properties, especially anti-Spodoptera, anti-Plutella, anti-Ostrinia anti-Mamestra; anti-Heliothis and anti-Agrotis properties, as the respective BTS02617A, BTS02618A, BTS02654B or BTS02652E crystals or

crystal proteins, or th BTS02618A protoxin or toxin. This invention also includes the <u>bTS02618A</u> gene and any insecticidally effective parts thereof, like the truncated <u>bTS02618A</u> gene. In this regard, the term "<u>bTS02618A</u> gene" as used herein means the gene isolated from the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain and hybridizing to the nucleotide sequence of SEQ ID No. 1 and any equivalent gene encoding a protoxin having substantially the same amino acid sequence and insecticidal activity as the BTS02618A protoxin and preferably containing the partial nucleotide sequences shown in SEQ ID Nos. 2 and 3, or the full sequence shown in SEQ ID No. 4.

This invention also is not limited to cotton plants transformed with the truncated <u>bTS02618A</u> gene. It includes any plant, such as tomato, tobacco, rapeseed, alfalfa, sunflower, lettuce, potato, corn, rice, soybean, <u>Brassica</u> species, sugar beet and other legumes and vegetables, transformed with an insecticidally effective part of the <u>bTS02618A</u> gene or an equivalent gene.

Nor is this invention limited to the use of <u>Agrobacterium tumefaciens</u> Ti-plasmids for transforming plant cells with an insecticidally effective <u>bTS02618A</u> gene part. Other known techniques for plant cell transformations, such as by means of liposomes, by electroporation or by vector systems based on plant viruses or pollen, can be used for transforming monocotyledons and dicotyledons with such a gene part.

Furthermore, DNA sequences other than those present naturally in the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains and encoding the BTS02618A protoxin and toxin can be used for transforming plants and bacteria. In this regard, the natural DNA sequence of these genes can be modified by: 1) replacing some codons with others that code either for the same or different, preferably the same, amino acids; 2) deleting or adding some codons; and/or 3) reciprocal recombination as described by Ge et al. (1991); provided that such modifications do not substantially alter the properties, particularly the insecticidal properties, especially anti-lepidoptera

properties, of the encoded, insecticidally effective portions of the BTS02618A protoxin (e.g., toxin). For example, an artificial bTS02618A gene or gene part of this invention, as described above, having a modified codon usage, could be used in certain circumstances instead of a natural insecticidally effective bTS02618A gene part in a bTS02618A chimeric gene of this invention for transforming plants.

Also, other DNA recombinants containing all or part of the bTS02618A gene in association with other foreign DNA, particularly the DNA of vectors suitable for transforming plants and microorganisms other than E. coli, are encompassed by this invention. In this regard, this invention is not limited to the specific plasmids containing the bTS02618A gene, or parts thereof, that were heretofore described, but rather, this invention encompasses any DNA recombinants containing DNA sequences that are their equivalent. Further, the invention relates to all DNA recombinants that include all or part of the bTS02618A gene and that are suitable for transforming microorganisms (e.g., plant associated bacteria such as other Bacillus thuringiensis strains, Bacillus subtilis, Pseudomonas, and Xanthomonas or yeasts such as Streptomyces cerevisiae) under conditions which enable all or part of the gene to be expressed and to be recoverable from said microorganisms or to be transferred to a plant cell.

Table 1.

Activity of CryI proteins towards several lepidopteran insect pests: + and - indicates the presence or absence of insecticidal activity, +/- indicates low activity (according to Van Frankenhuyzen (1993)), NA indicates no data available, the protein BTS02618A is abbreviated as 2618A (data of Van Frankenhuyzen (1993) and this invention (for A. ipsilon and 2618A)).

	2618A	IAb	IAc	IB	IC	IF
S.exiqua	+	+/-	-	-	+	+
S.littoralis	+	-	-	-	+	NA
H.virescens	+	+	+	-	+/-	+
A.ipsilon	+	NA	-	NA	NA	NA
O.nubilalis	+	+	+	NA	NA	+
P.xylostella	+	+	+	+	+	NA
M.brassica	+	+	-	-	+	NA

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